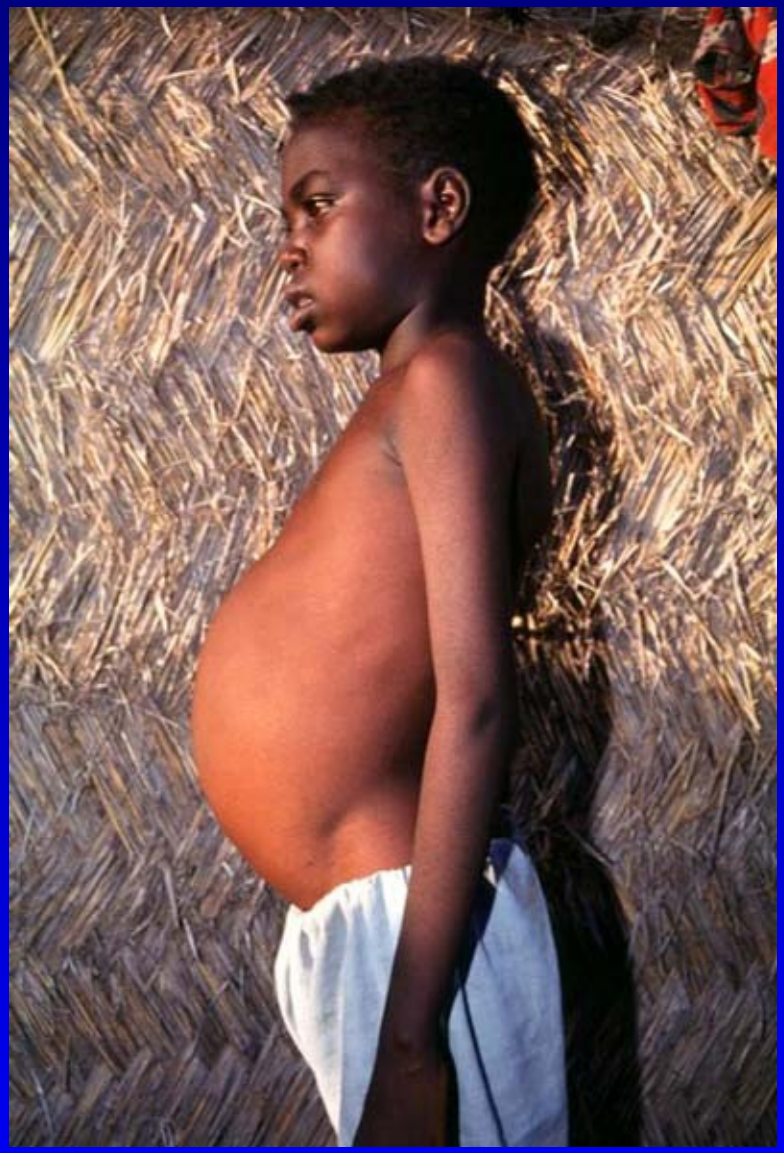


GLOBAL MEDICINE
30 Jan - 10 Feb 2005

USAF Course Number:
B30ZY48X0-000

Leishmaniasis

hology, Epidemiology, and Clinical Disea

Alan J. Magill M.D., FACP

COL / MC / USA

Director, Division of Experimental Therapeutics

Walter Reed Army Institute of Research

Silver Spring, MD, USA

William Leishman & Charles Donovan

The Leishmaniases

- **A diverse group of protozoan parasites**
- **Different clinical manifestations / syndromes**
- **Zoonosis**
  - **Sand fly insect vector**
  - **Mammalian reservoir(s)**
  - **Man is incidental host (usually)**
    - **Indian *L. donovani* VL and**
    - ***L. tropica* CL are exceptions**

3 Major Clinical Syndromes

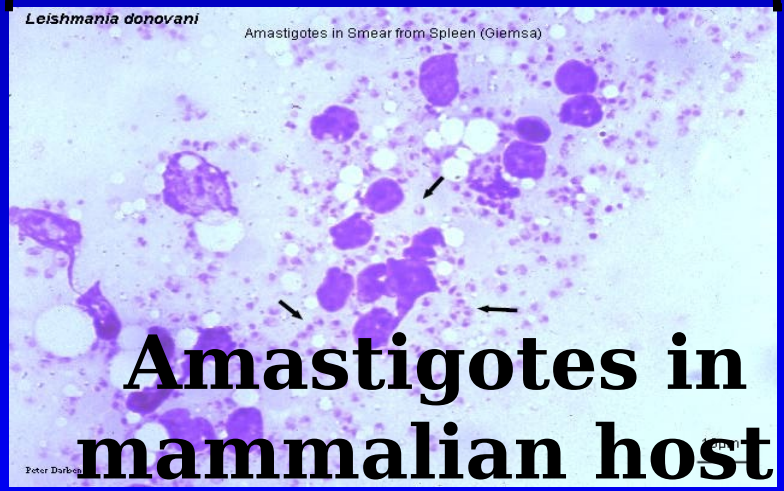
Visceral Leishmaniasis

Cutaneous Leishmaniasis

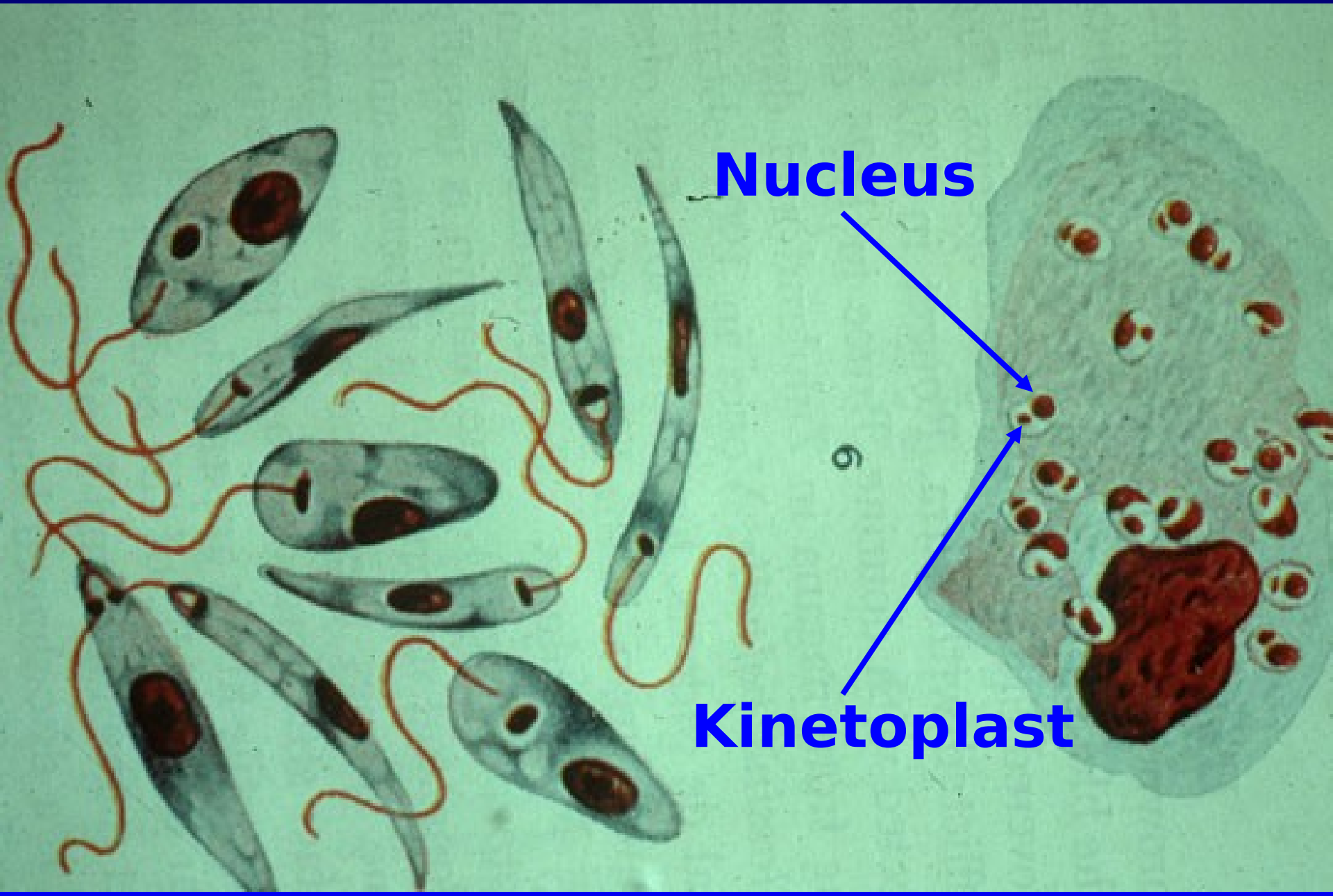
Mucosal Leishmaniasis

Parasite Morphology

Promastigotes in sand fly



Amastigotes in mammalian host


Nucleus
Kinetoplast

The Epidemiological Triangle

Enzootic Cycle

Sand fl

Mammali Reservoi

Incidental Host

Man and his Activities

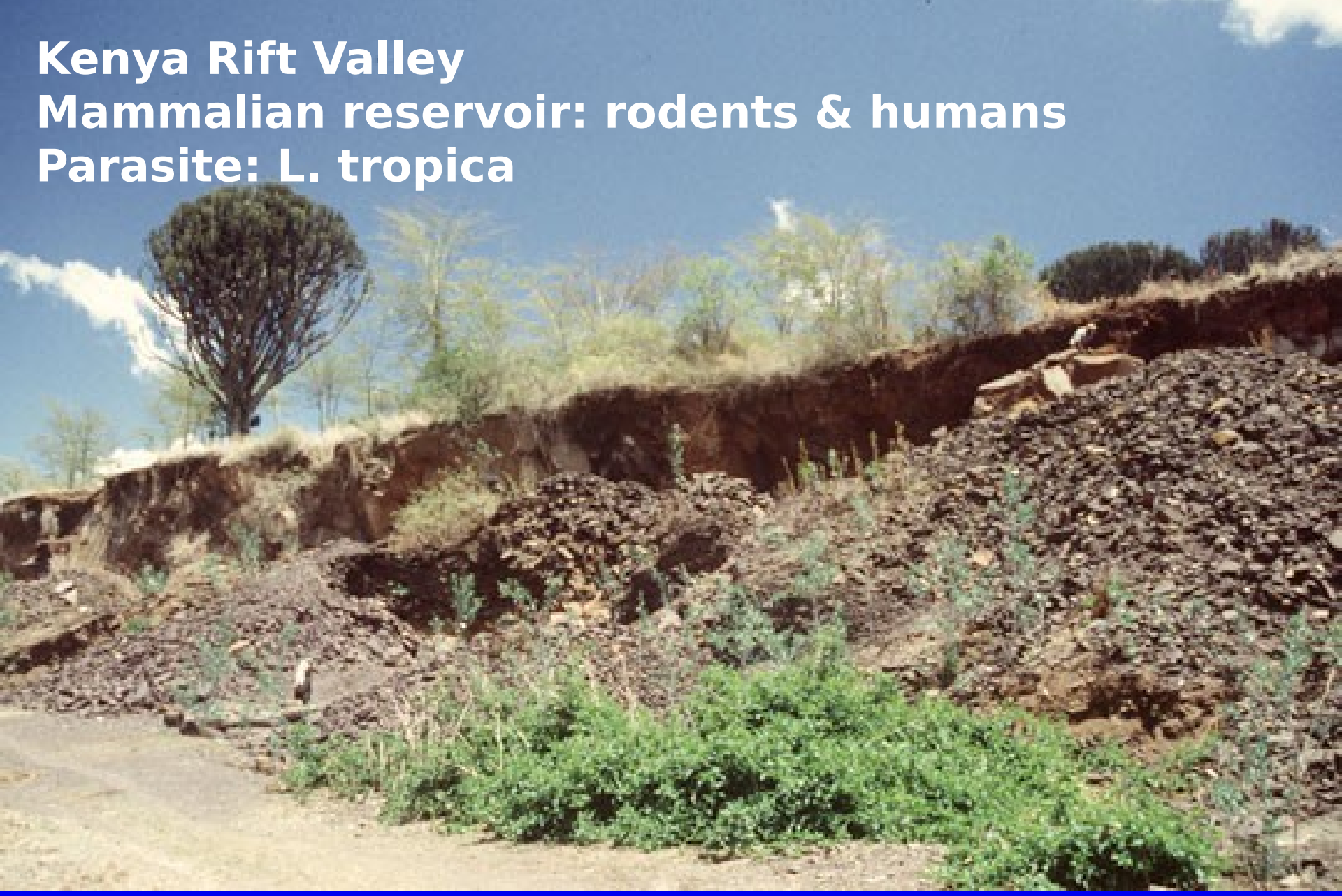
Modes of Transmission

- **Sand fly insect vector**
- **Parenteral**
  - **Blood transfusion**
  - **Intravenous Drug Abuse (needle sharing)**
- **Congential**
- **Occupational (laboratory needle stick)**
- **Sexual**
- **Person to person?**

Amazon Rainforest
Mammalian reservoir: rodents
Parasite: L. braziliensis

Brazilian peri-urban
Mammalian reservoir: domestic dogs
Parasite: L. chagasi

Kenya Rift Valley
Mammalian reservoir: rodents & humans
Parasite: L. tropica



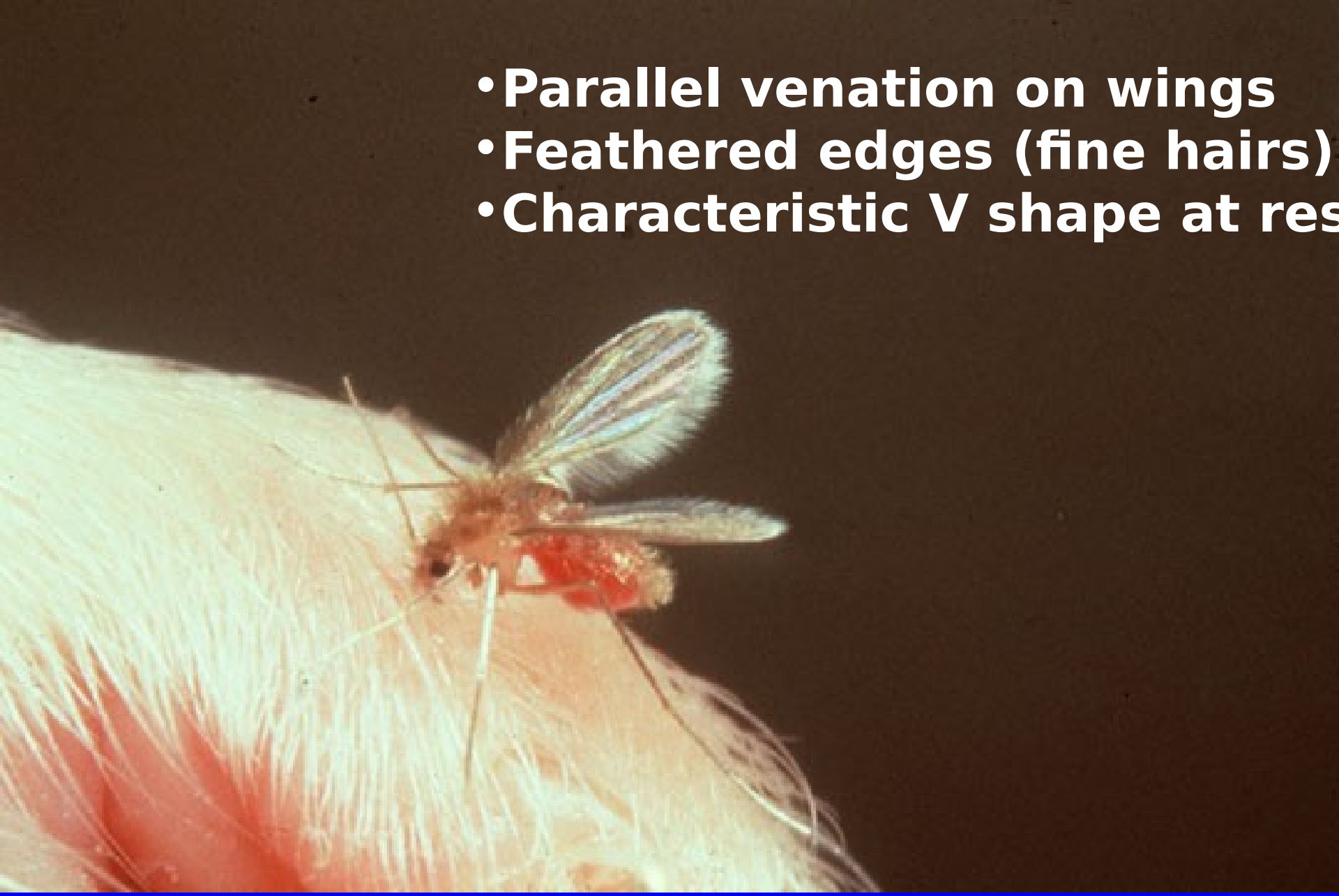
Gangetic plains, Bihar, India
Mammalian reservoir: humans
Parasite: L. donovani

Psammomys obesus

Middle East
Enzootic cycle
Mammalian Reservoi
Small desert rodents
Parasite: *L. major*

Chenopods

***L. major* enzootic cycle**


•Parallel venation on wings
•Feathered edges (fine hairs)
•Characteristic V shape at res

Cutaneous Syndromes

- **Cutaneous leishmaniasis**
  - **Localized, ulcerative**
  - **Nodular**
  - **Other: Psoriaform, verrucous, plaque, macules**
- **Diffuse cutaneous leishmaniasis**
- **Leishmaniasis recidivans**
- **Mucosal leishmaniasis**
- **Post Kala Azar Dermal Leishmaniasis (PKDL)**

Cutaneous Leishmaniasis

- Cutaneous leishmaniasis
  - Localized, ulcerative
  - Nodular
  - Other: Psoriaform, verrucous, plaque, macules
- Self healing
- Incubation period of days to years
- Slowly enlarging nodule at bite site
- Crust forms centrally leading to ulceration with raised margins
- Healing leaves a hypo-pigmented and depressed scar
- Characteristic but inconstant clinical differences between species

DATE
4
3
2
1

Mucosal Leishmaniasis

- **“Espundia”**
- **Nodular and ulcerative lesions of the oral-nasal-pharyngeal mucosal surfaces**
- **Days to years (43!) following primary infection with *L. braziliensis***
- **Healed scar of primary CL infection usually present**
- **Nasal septum lesion or horseness often first symptom or sign**

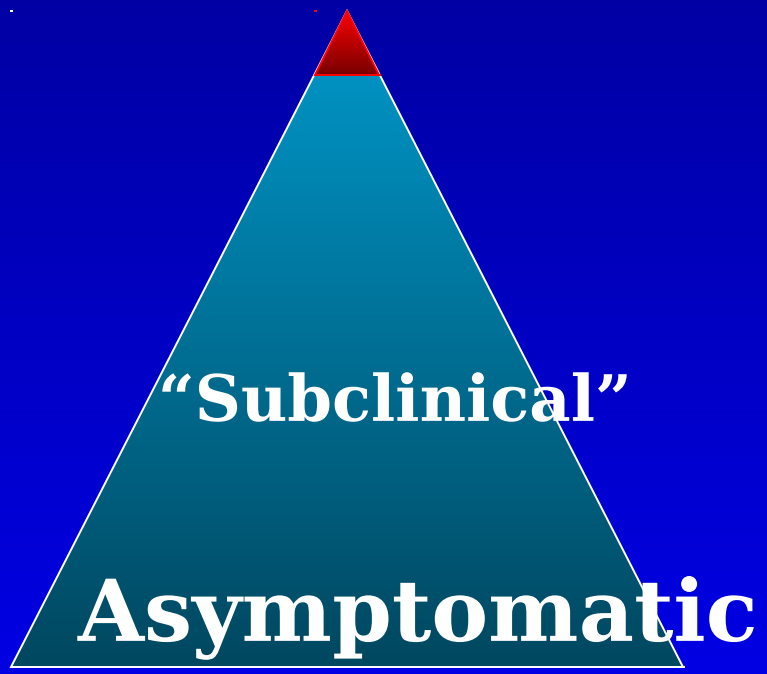
Visceral Leishmaniasis

- ***Classic "pentad"***
  - **Fever**
  - **Cachexia**
  - **Splenomegaly**
  - **Pancytopenia**
  - **Hypergamma-globulinemia**

Mediterranean Kala-azar
L. infantum

Visceral Leishmaniasis Disease Spectrum

1-3% with overt VL



- **"Subclinical" Syndromes**
  - **Chronic systemic illness**
  - **Acute febrile illness**
- **Risk factors for progression**
  - **Malnutrition**
  - **Immunosuppression (AIDS)**
  - **Genetic?**
- **Cause of death**
  - **Measles**
  - **Pneumonia**
  - **TB**
  - **dysentery**

VL Case Report: History

- 25 yo AD USAF male presents with CC of increasing difficulty completing a 2 mile run
- ROS reveals fatigue, night sweats, 30 pound weight loss over 3-4 months
- Lived in Spain from March 93 to June 94
- One visit to MD in June 94 for abrupt onset of fever, malaise
  - 5 X increase AST.
  - Resolved w/o specific dx or intervention

VL Case Report: Physical Exam and Lab

- Thin, chronically ill appearing male
- Afebrile
- Marked splenomegaly (30 cm below costal margin)
- Anemia (Hb / Hct = 7.7 / 23)
- Thrombocytopenia (WBC = 2600)
- Marked increase in total protein to 18 .4 g / dl (NR = 6.4 - 8.2 g / dl)

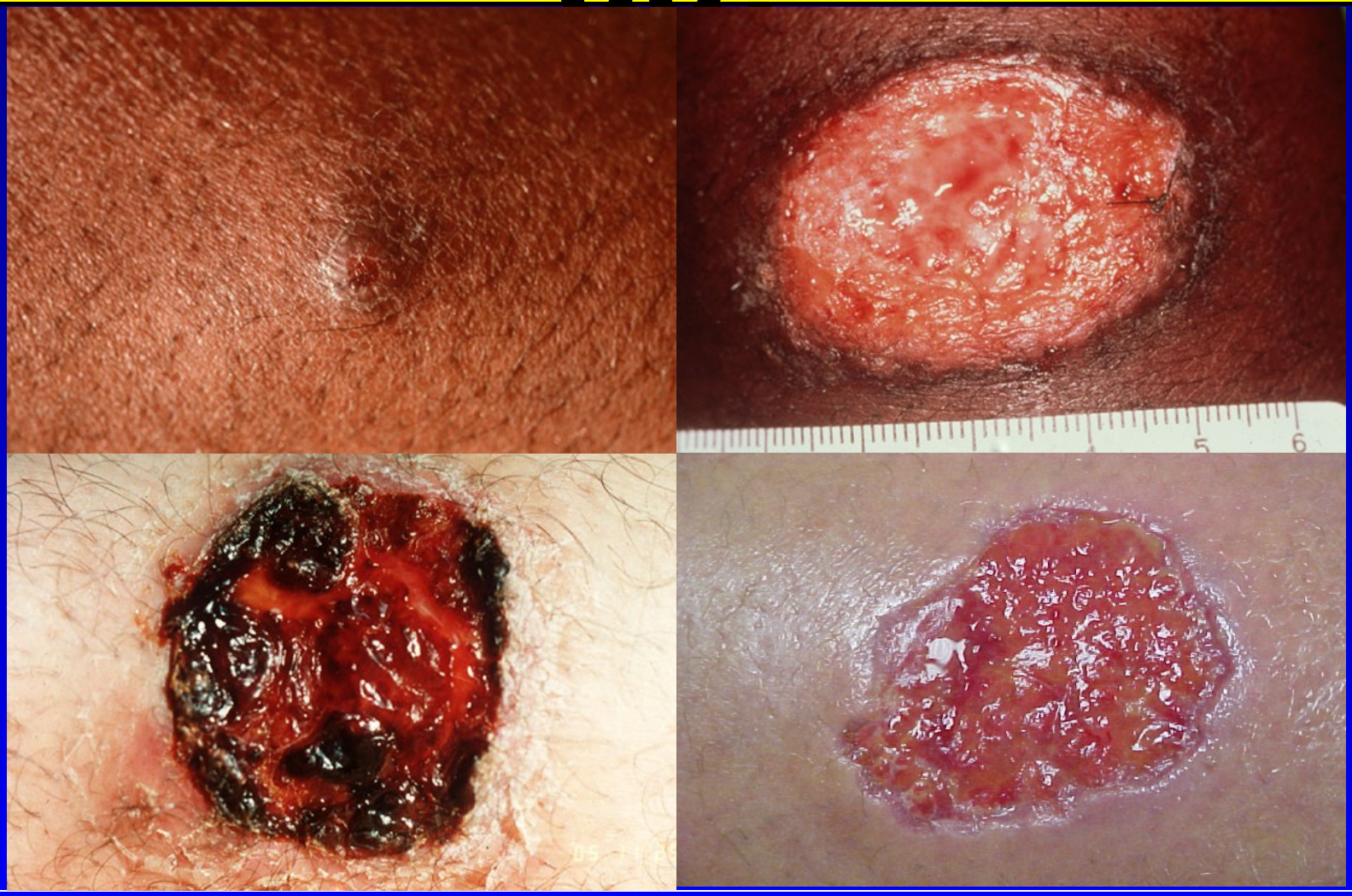
Clinical Presentation of VL in the Immunocompetent,Well Nourished Adult

- ***Most and Levietes. 1947. Medicine. 26:221***
  - **N=30, US military in WWII**
  - **Incubation period: 3 weeks to 33 months**
  - **Symptom onset to definitive diagnosis, mean 10 weeks (range 2 -26 weeks)**
  - **Abrupt onset of fever and chills - 96%**
  - **Splenomegaly in 90% and hepatomegaly in 73% on presentation**
  - **Parasitologic diagnosis**
    - **21 of 49 (43%) smears from bone marrow aspirations in 29 patients were (+)**
    - **8 0f 29 (28%) were not confirmed by bone marrow**
    - **18 of 18 (100%) splenic aspirates smear and culture (+)**

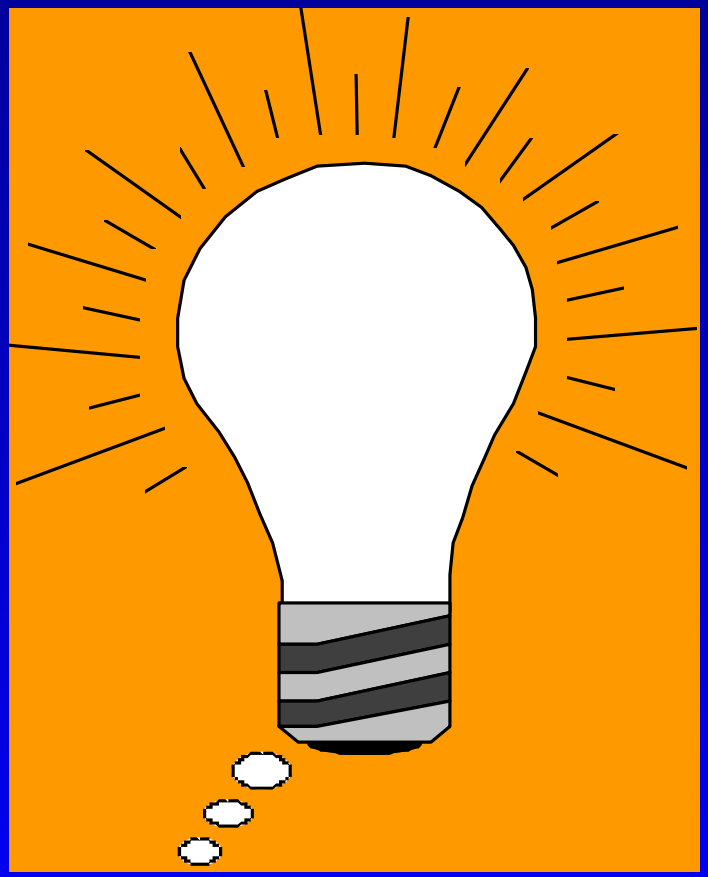
Leishmaniasis as an OI in the Immunocompromised Host

- ***Primary disease***
  - **HIV, SLE, Hodgkin's disease, acute leukemia**
- ***Immunosuppressive drugs***
  - **Cyclosporin A, prednisone, cyclophosphamide, azathioprine**
- ***Organ transplantations***
  - **Kidney, Heart, Liver**

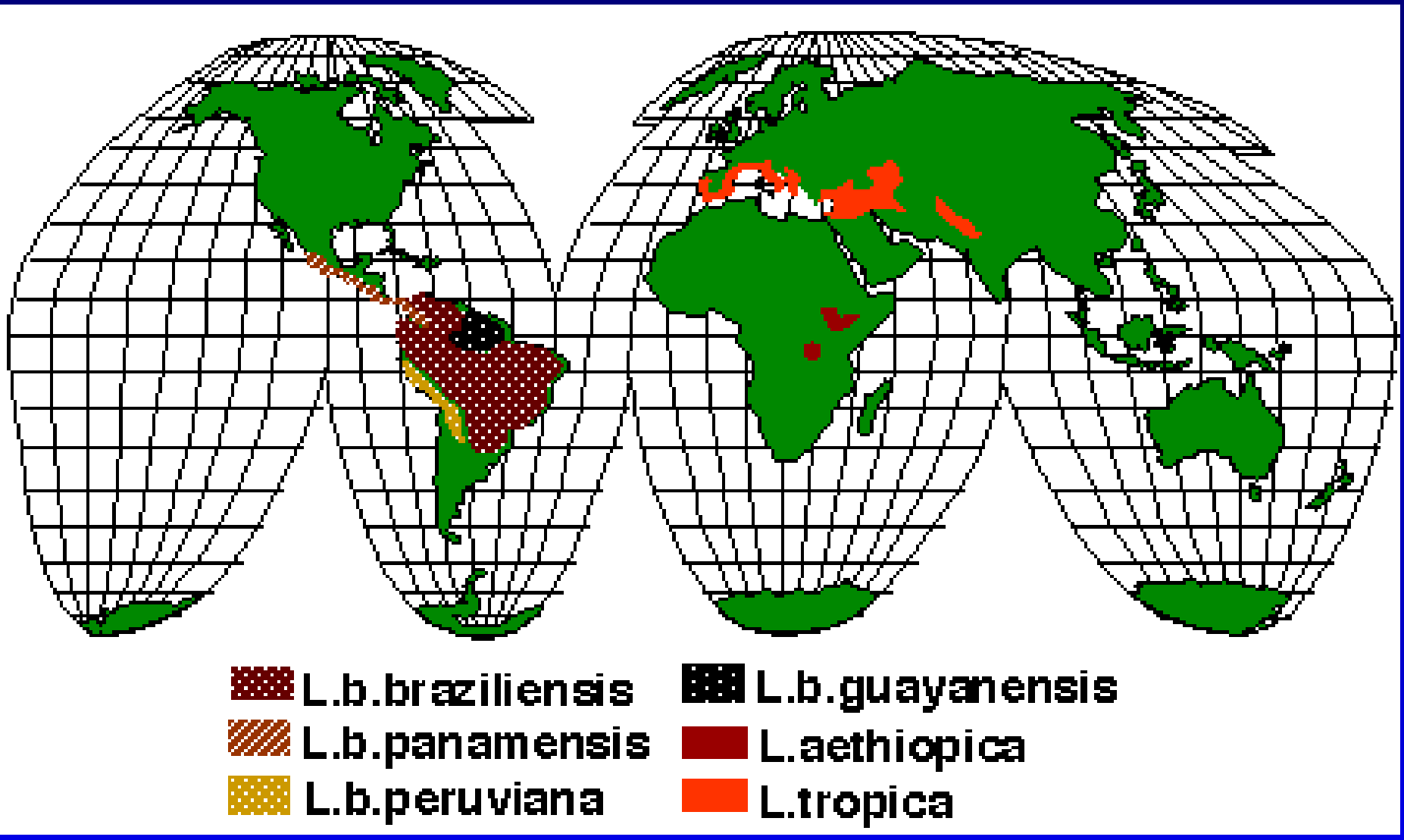
So now what do I do?



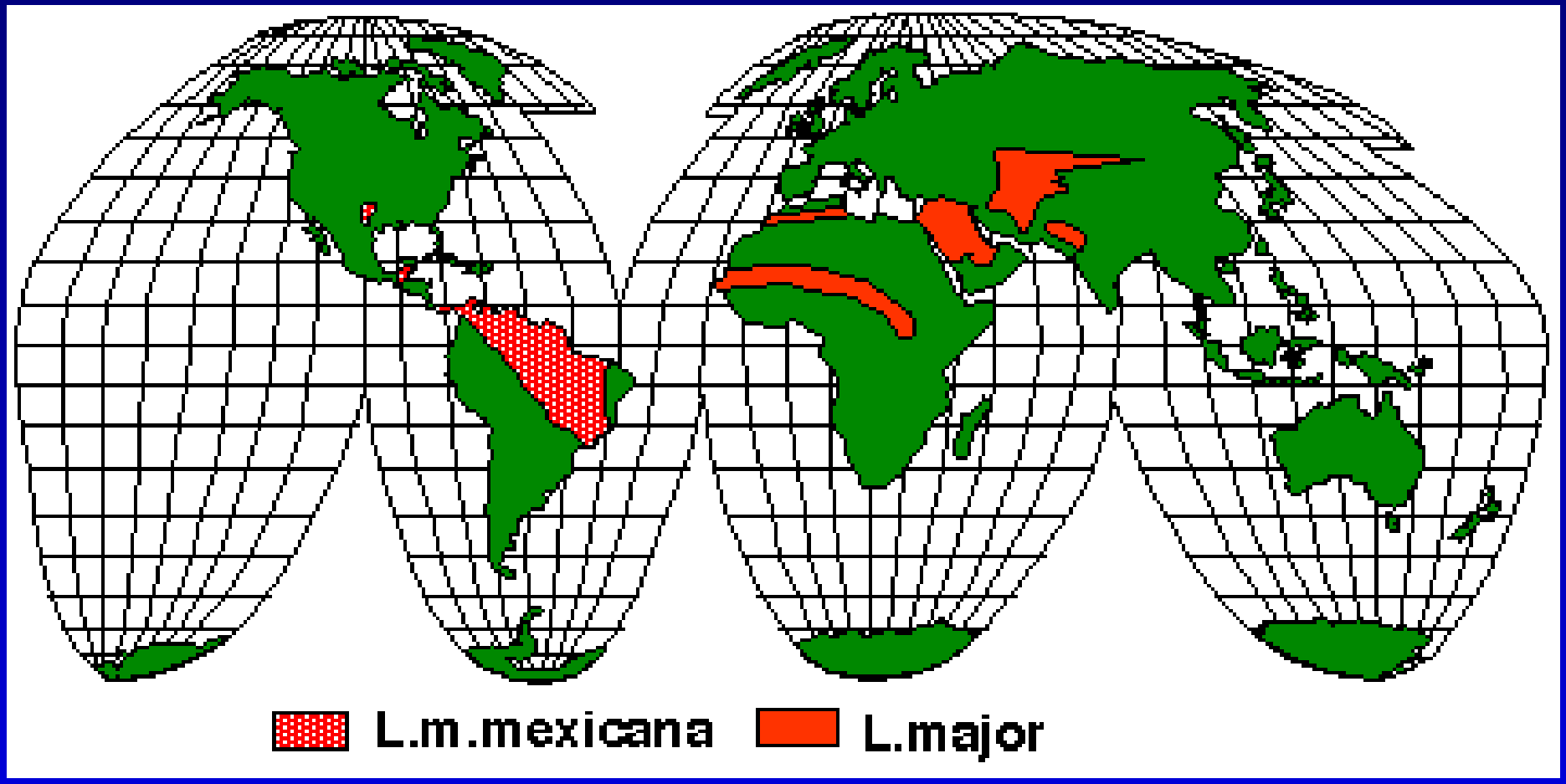
Diagnosis



- Travel history
- Deployment history
- Birth history
- Residence history
- Occupational exposure
- Blood transfusions
- Congenital



Courtesy of http://www.icp.ucl.ac.be/~opperd/parasit



Courtesy of http://www.icp.ucl.ac.be/~opperd/parasi

Diagnostic Approaches

- **Clinical / epidemiological diagnosis**
- **Parasitologic Diagnosis**
  - **Amastigotes on a slide or in tissue**
  - **Promastigotes in culture**
  - **Molecular**
- **Immunological Diagnosis**
  - **Serology**
  - **Delayed type hypersensitivity (DTH) via skin test**

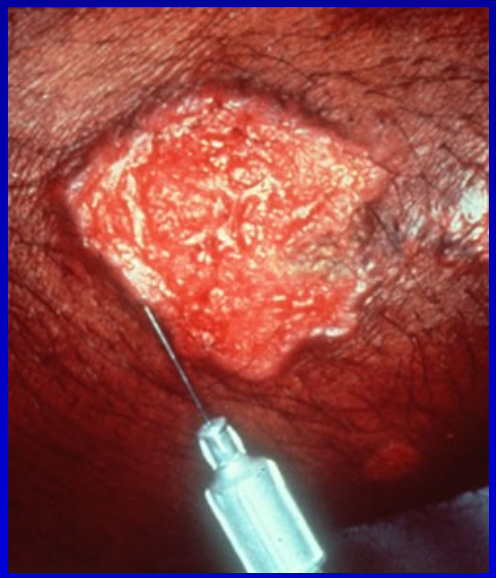
Where and how do I sample?

- Site preparation
- How to sample?
  - Scraping
  - Aspirate
  - Punch bx
- Where to sample
  - Margin versus center of ulcer

Site Preparation

- **Ulcerative lesions**
  - **Lidocaine, debridement, clean & dry ulcer base**
  - **May lightly use scrub brush**
  - **Remove all surface exudate**
- **Nodular lesions**
  - **Soap and water**
  - **± lidocaine for slit-skin**

How to get the specimens?

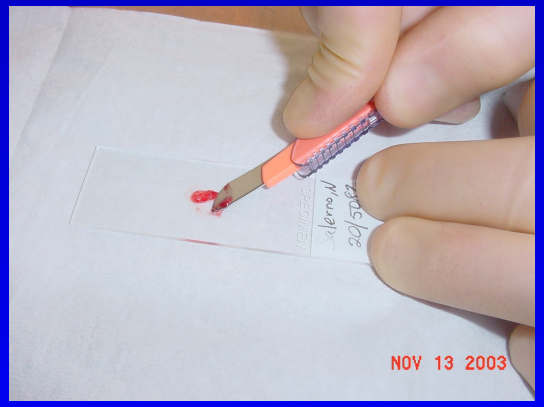


Scrape ulcer base with No. 10 scalpel blade

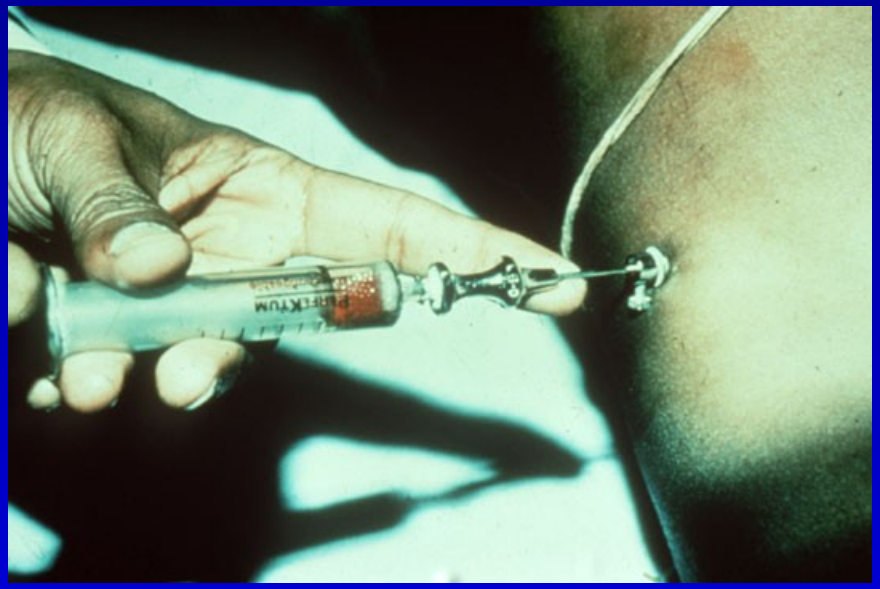
Aspirate the lesion 2-3 mm with sterile saline in hub

Skin punch bx

Diagnosis: Scraping Method

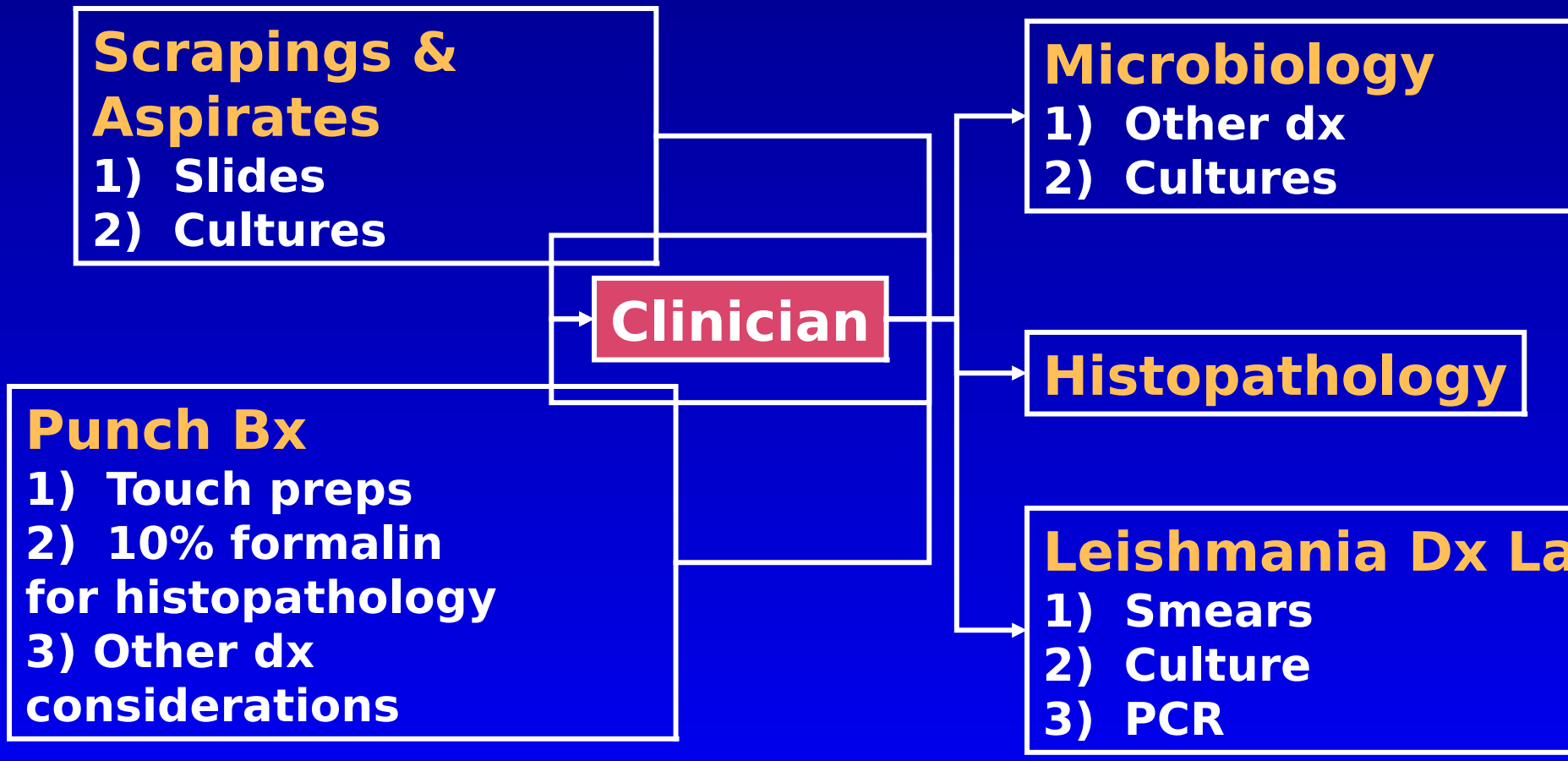


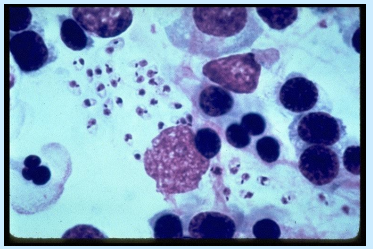
How to get the specimens?



Splenic aspiration
Sterile saline in hub of a syringe

See the amastigote!





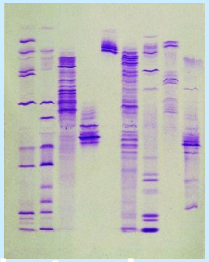
Touch preps

Giemsa staining

Lesion biopsy or aspirate

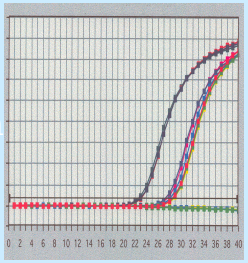
Schneiders, MMI, NNN culture media

Promastigotes in culture



Electrophoresis for speciation

PCR



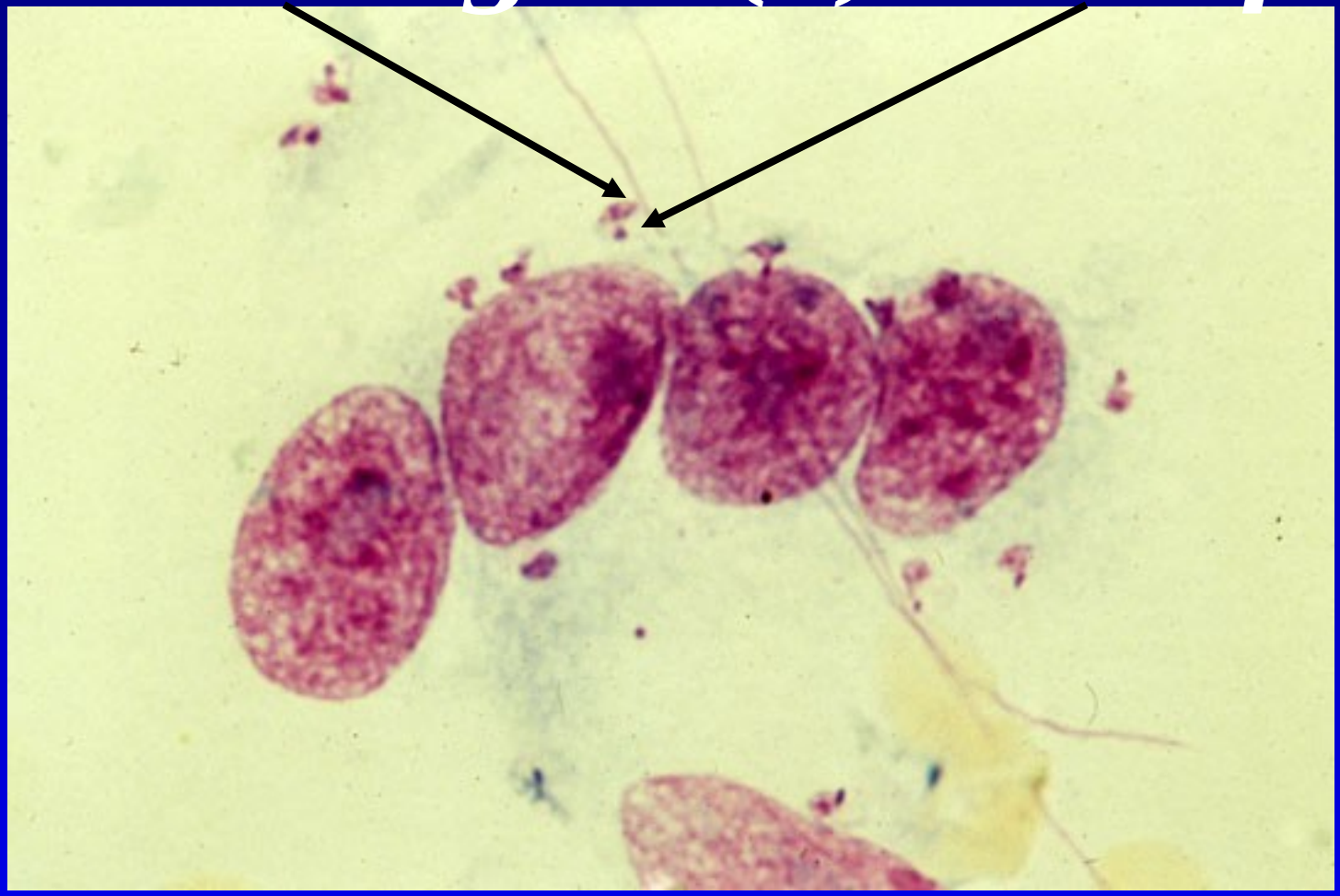
L. major
L. major
L. V. panamensis

Negative control

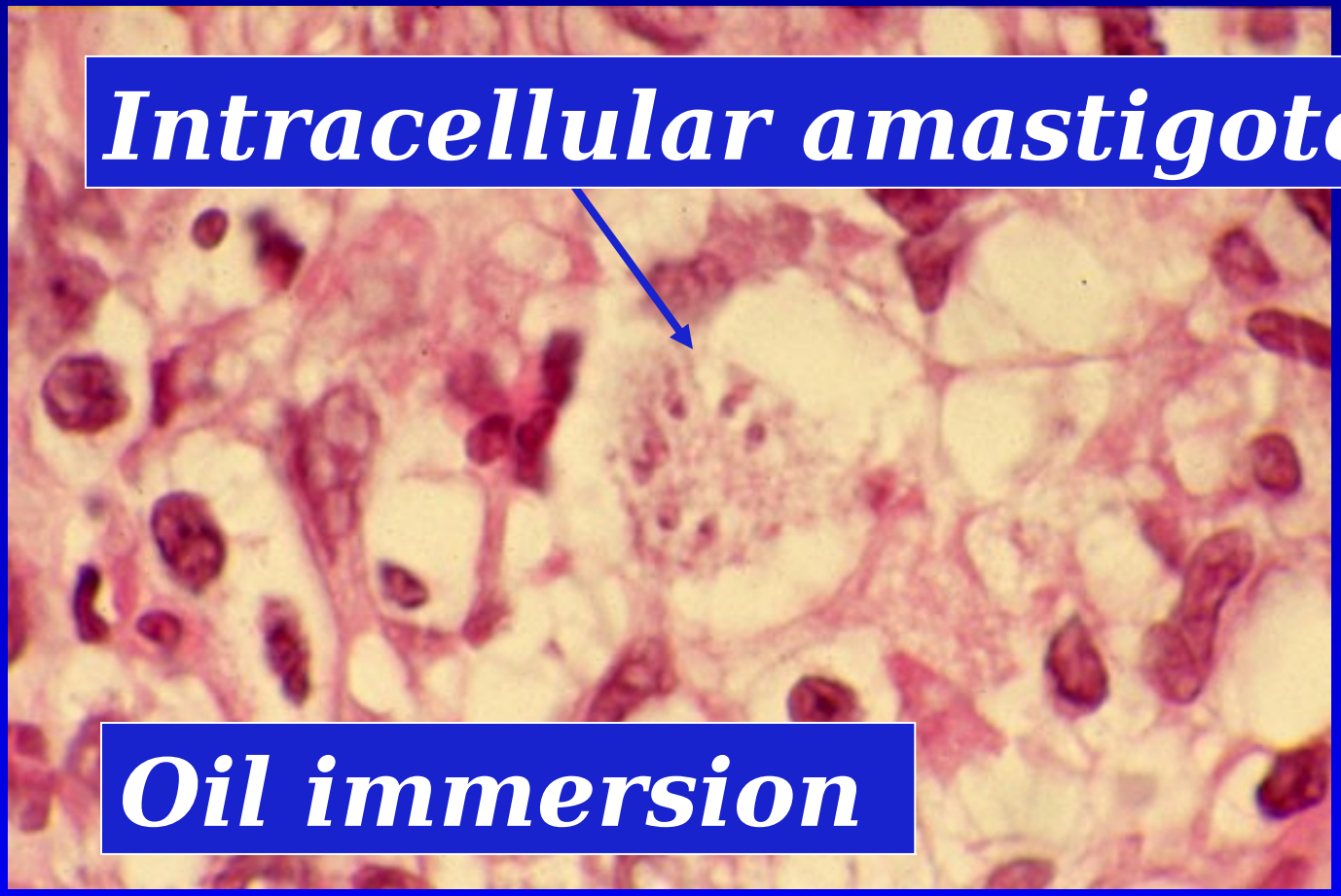
rRNA Probe for Genus Level Identification

Processing of diagnostic material for Leishmaniasis evaluation

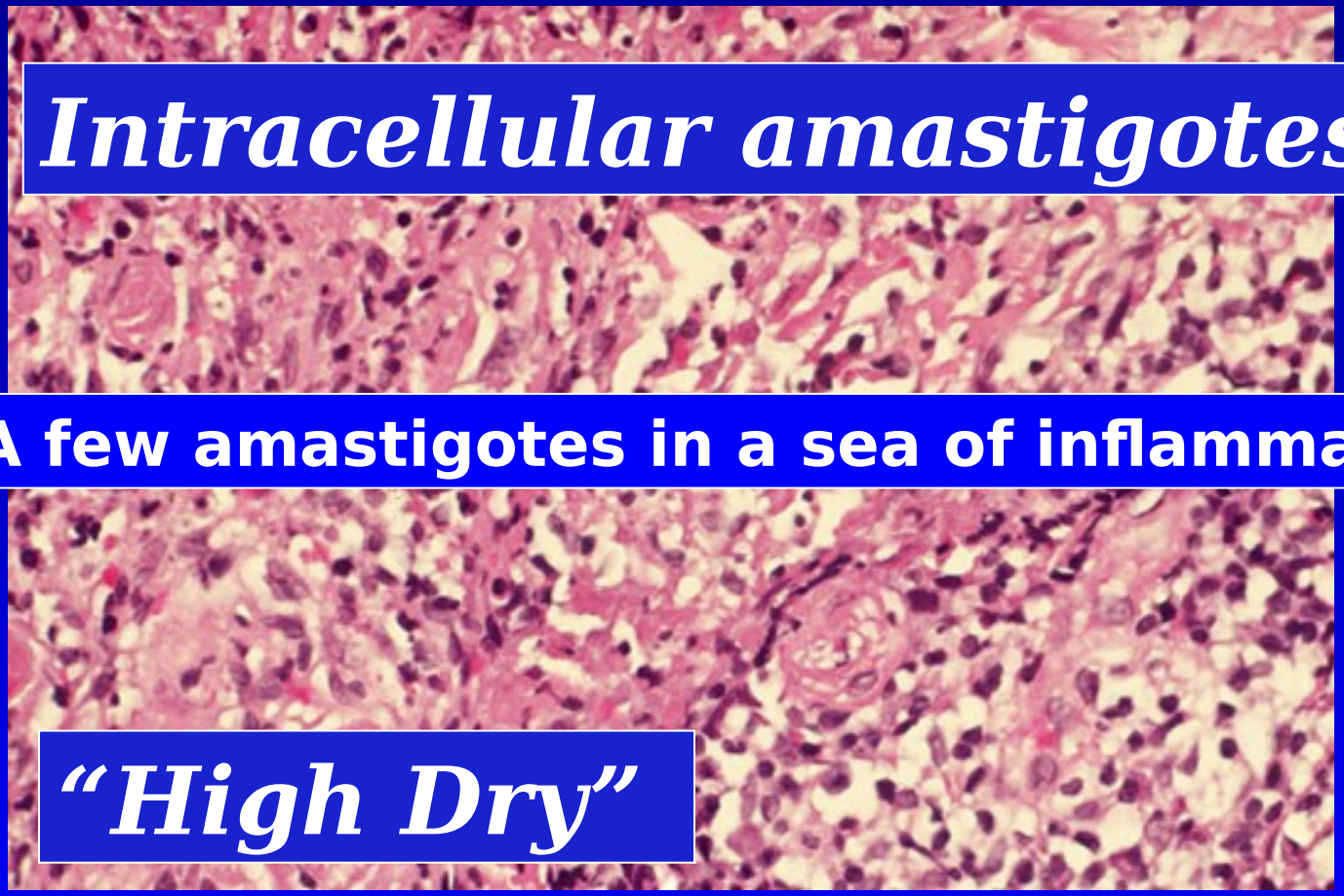
Amastigote(s) Kinetoplast



Histopathology: H&E / Giemsa



Histopathology: H&E / Giemsa



Intracellular amastigotes ?

"A few amastigotes in a sea of inflammatory cel

"High Dry"

Histopathology

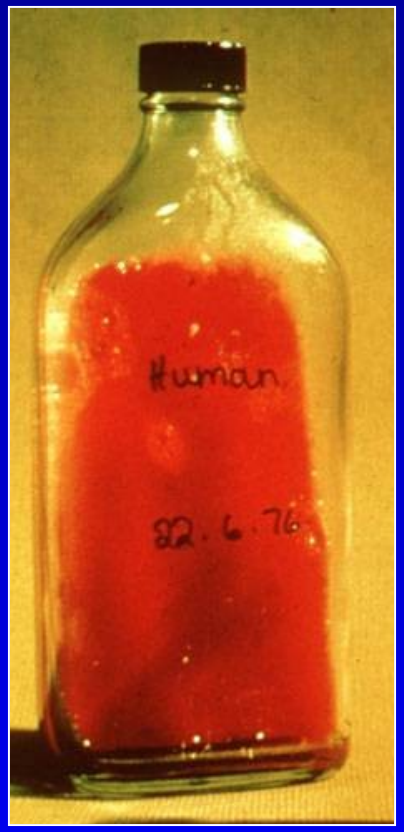
- **Most North American pathologists do not routinely review slides under oil immersion**
- **Lack of experience looking for Leish**
- **Cuts too thick (5-6 µm) vs. 1 µm for Leish**
- **Review slide for 1 hour under oil to call negative**
- **Histopathology least sensitive of any method**
  - **As low as 12% in one study**
- **Useful to exclude other diagnoses and describe histopathology**

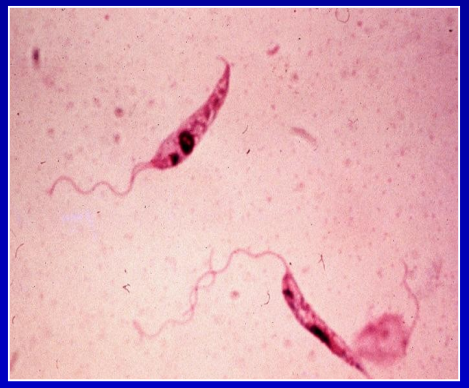
Nodular Presentations

- Slit-skin smears of nodule or nodule margin
- Punch biopsies?
- Not uncommon presentation
  - Part of the natural hx
  - 10% persistent

Leishmania Culture

- "Science" versus "art"
- Complex lab skills
- Asking the intracellular amastigote to transform into a promastigote and expand
- Parasite species growth differences

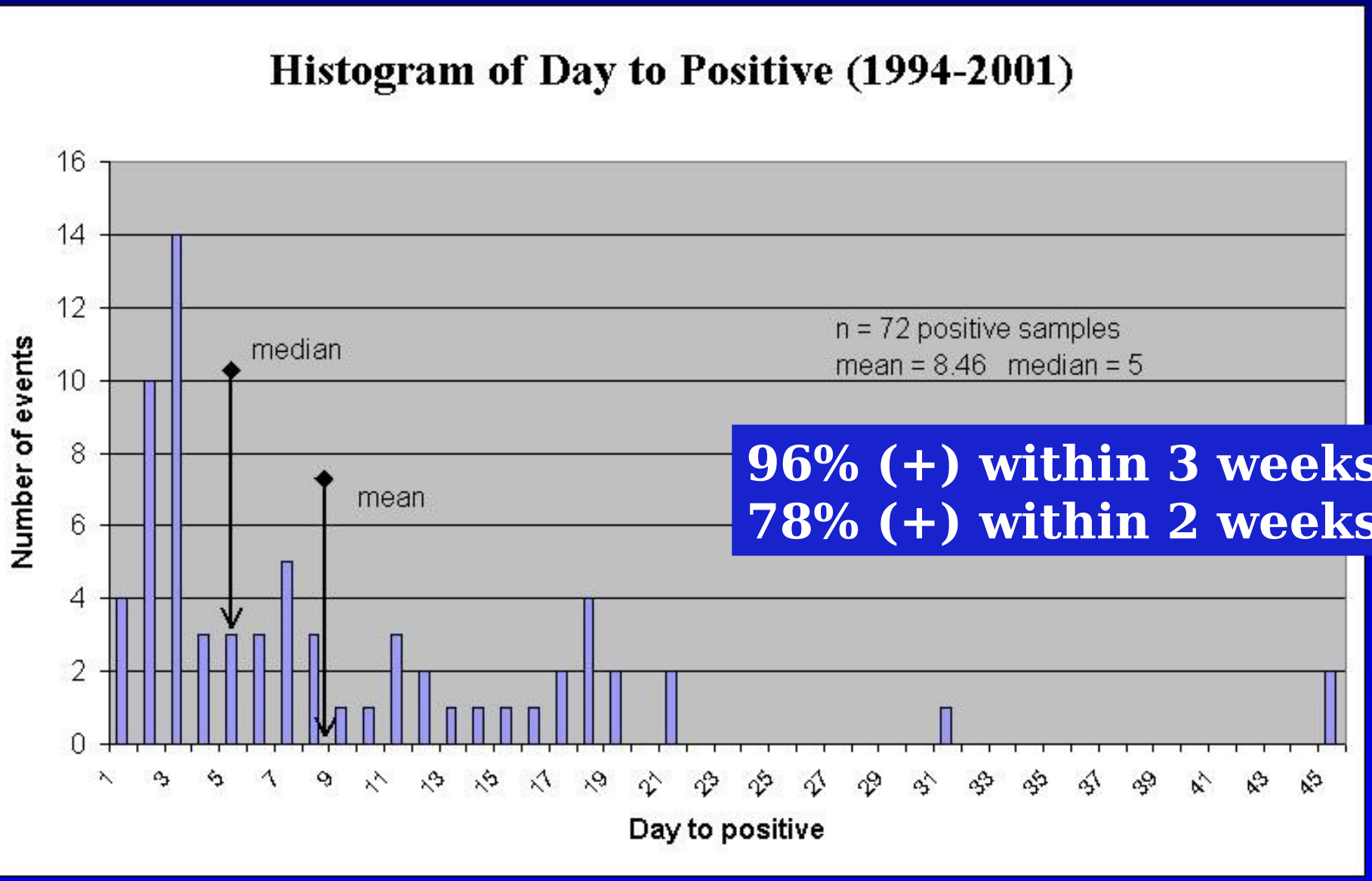




Association between culture results and concentration of amastigotes on thin smears

Amastigotes / 400 oil immersion fields	Culture results No. positive / total (%)
0	42 / 153 (27)
1-19	36 / 50 (72)
20-199	43 / 53 (81)
200-399	27 / 32 (84)
400-799	42 / 46 (91)
800-1,999	12 / 13 (92)
≥2,000	15 / 15 (100)

Navin TR et al. Am J Trop Med Hyg 1990 42(1),


Histogram of Day to Positive (1994-2001)
Number of events
Day to positive
median
mean
n = 72 positive samples
mean = 8.46 median = 5
96% (+) within 3 weeks
78% (+) within 2 weeks

PCR

- **Genus specific and / or species specific**
- **Sensitivity / specificity with different species, targets, probes, specimens, geographic locations**
- **Who does it?**
- **Where is it done?**

PCR

- **Real-time PCR assay**
  - **Diagnosis within hours**
- **Currently available at WRAIR**
- **Potential to project into Iraq**

Current Experience with PCR

- 347 samples tested from OIF soldiers
- PCR 97% (335/347)
- Microscopy 76% (262/347)
  - AFIP expert review
- Culture 78% (270/347)
- PCR statistically more sensitive than either alternative technique ($p<0.0005$)

Parasitologic Diagnosis Summary

- Sample youngest or “most active” lesion(s)
- Use more than one technique on more than one sample
  - 5 smears from dermal scrapings of different sites on the ulcer(s)
  - 3-5 cultures
  - PCR is good!
- Success correlates with parasite burden
- Can never exclude the diagnosis with only parasitological methods

Immunologic Diagnosis

- **Serology (detection of antibodies)**
  - **Not currently useful in CL**
  - **Can be useful in VL**
    - **DAT, ELISA, rK39**
- **DTH - skin testing**
  - **No product available or licensed in the USA**
  - **Useful adjunct in oligoparasitic syndromes such as ML, CL, resolved VL,**

rK39 based serologic tests

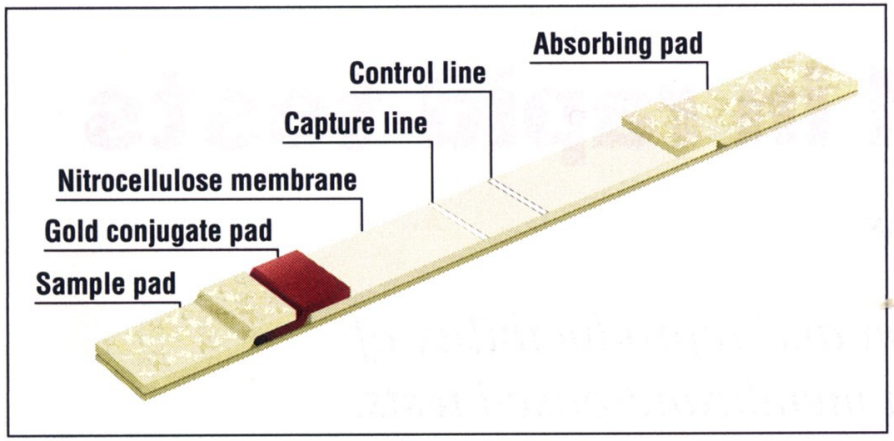


Figure 1. Construction of a lateral-flow rapid test.

- Kalazar Detect™
- FDA cleared via 510K process on 06 May 2003
- InBios International Inc. Seattle, WA
- Detects Ab to rK39 Ag in VL
- > 95 % sensitivity
- Peripheral blood

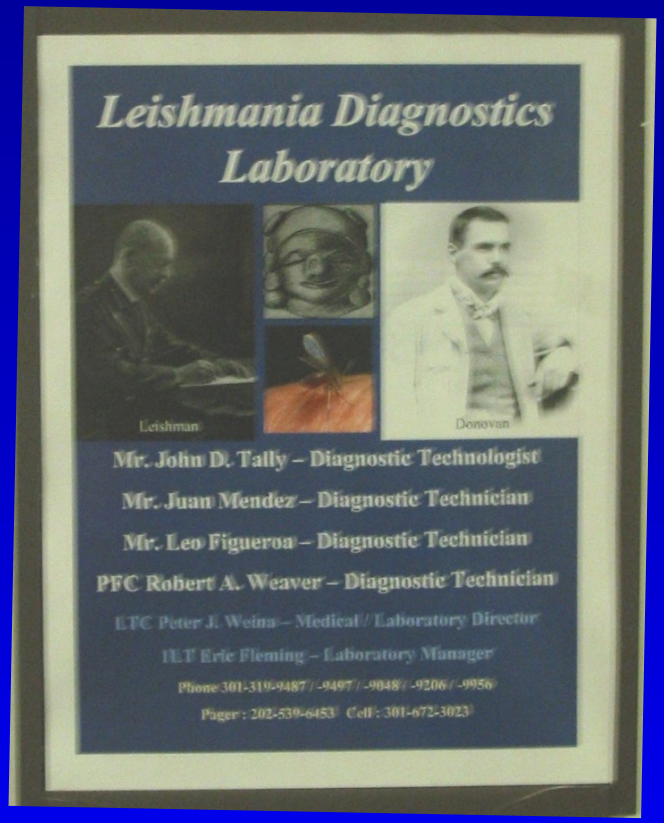
Does it matter what the Leishmania species is?

- Rx or not to Rx?
  - L. braziliensis or L. panamensis
- Choice of drug Rx?
- Duration of Rx?
  - L. major or L. tropica
- Dose?

Species identification

- Morphology on slide? Not for routine use
- Isoenzyme analysis
  - Comparison of unknown to reference standards
  - Requires cultured promastigotes
  - Patients usually treated before result is known
- Mab
- PCR

WRAIR Leishmania Diagnostic Laboratory



- LTC(P) Peter Weina, Director
- CAP & CLIP certified
- 301-319-9956, 7733, 9497, 9487

WRAIR Capabilities

- Microscopic review of slides
- In vitro culture
- Isoenzyme species identification if culture (+)
- PCR

WRAIR “mail out” diagnostic kit

- Instructions
- Culture media
- Return mailing box with permits
- Vials with EtOH for PCR
- Vials with 10% formalin for histopathology
- Slides, slide holders, punch bx, gauze, etc.

CDC Diagnostic Services

- Frank Steurer, 770-488-4475
- Microscopy
- Culture with isoenzyme analysis
- Serologic testing (IFA)
- No Mab or PCR testing

So how do I treat this?

Amastigotes seen, kinetoplast confirmed

Drug Therapy: Basic Principles

Success of SbV dependent on functional CD4(+) T cells

Lack of RDBPC trials

- **Different endpoints**

  Clinical cure = resolution of signs and symptoms of disease

  Parasitological cure = eradication of parasites

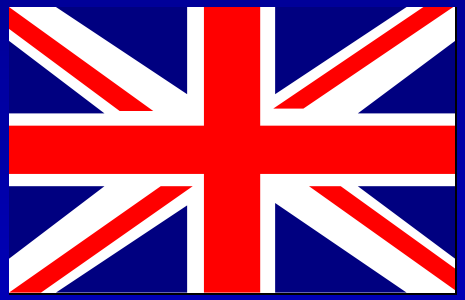
Geographic Variability

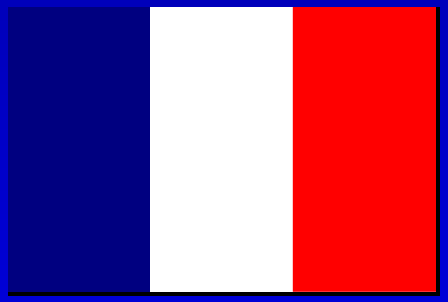
- **Heterogenous populations**
- **Complex disease**

Drug Therapy: Current Status (2006) in USA

- **AmBisome® (Liposomal amphotericin B)**
  - **Approved for Rx of VL by the FDA**
- **Pentostam® [pentavalent antimony, (SbV]**
  - **Available under an IND**
    - **Military personnel**
      - **Walter Reed Army Medical Center**
      - **Brooke Army Medical Center**
    - **Civilian personnel - CDC**

Drug Therapy: SbV





- **Pentostam® (sodium stibogluconate, Glaxo-Wellcome, UK)**
  - **100 ml bottles at 100 mg Sb / ml**
  - **Traditionally used in Anglophone countries**
- **Glucantime® (meglumine antimoniate, Rhone-Poulenc, France)**
  - **5 ml ampules at 85 mg Sb / ml**
  - **Traditionally used in Francophone, Spanish and Portuguese speaking countries**

20 mg / kg / day X 20 days for LCL
20 mg / kg / day X 28 days for VL and ML

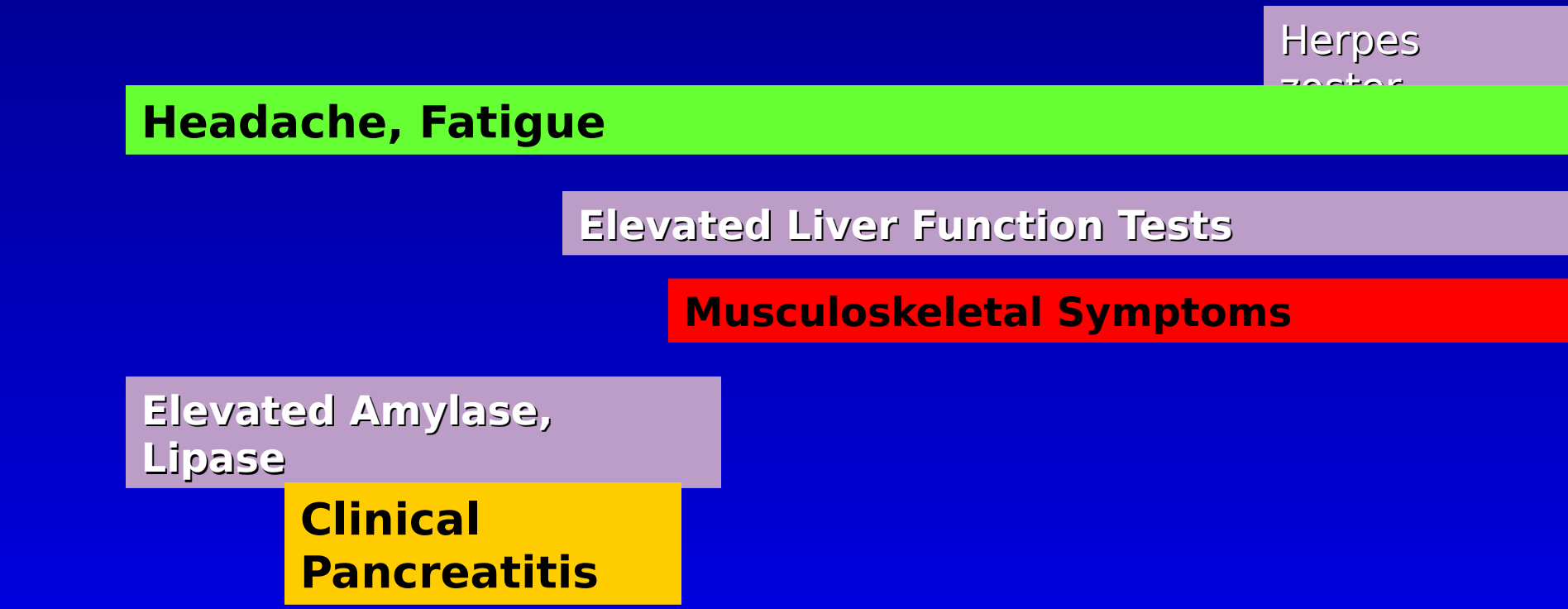
Pentostam®: Side Effects[1]

- **Symptoms:**
  - **Headache, somnolence, fatigue**
  - **Anorexia, nausea, vomiting, abdominal pain**
  - **Large joint arthralgias and stiffness, myalgias**

Pentostam®: Side Effects[2]

- **Signs**
  - **Mid-epigastric tenderness**
  - **Decreased range of motion of large joints**
- **Special Tests**
  - **Increased ALT / AST**
  - **Increased serum lipase / amylase**
  - **Anemia, thrombocytopenia, granulocytopenia**
  - **T wave flattening, nonspecific ST segment changes**
  - **Prolonged QTc interval on ECG**

Sodium stibogluconate:
Timeline for Common Side Effects



Drug Therapy: Current Issues

- **Worldwide**
  - **Increasing SbV clinical failures in Indian VL**
    - **Primary drug resistance**
    - **Noncompliance**
    - **Sub-optimal regimens prescribed and used**
  - **Oral Miltefosine and IV paromomycin for Indian VL**
- **USA, military and travellers**
  - **No efficacious oral agents for CL**
  - **No FDA approved treatment for CL**

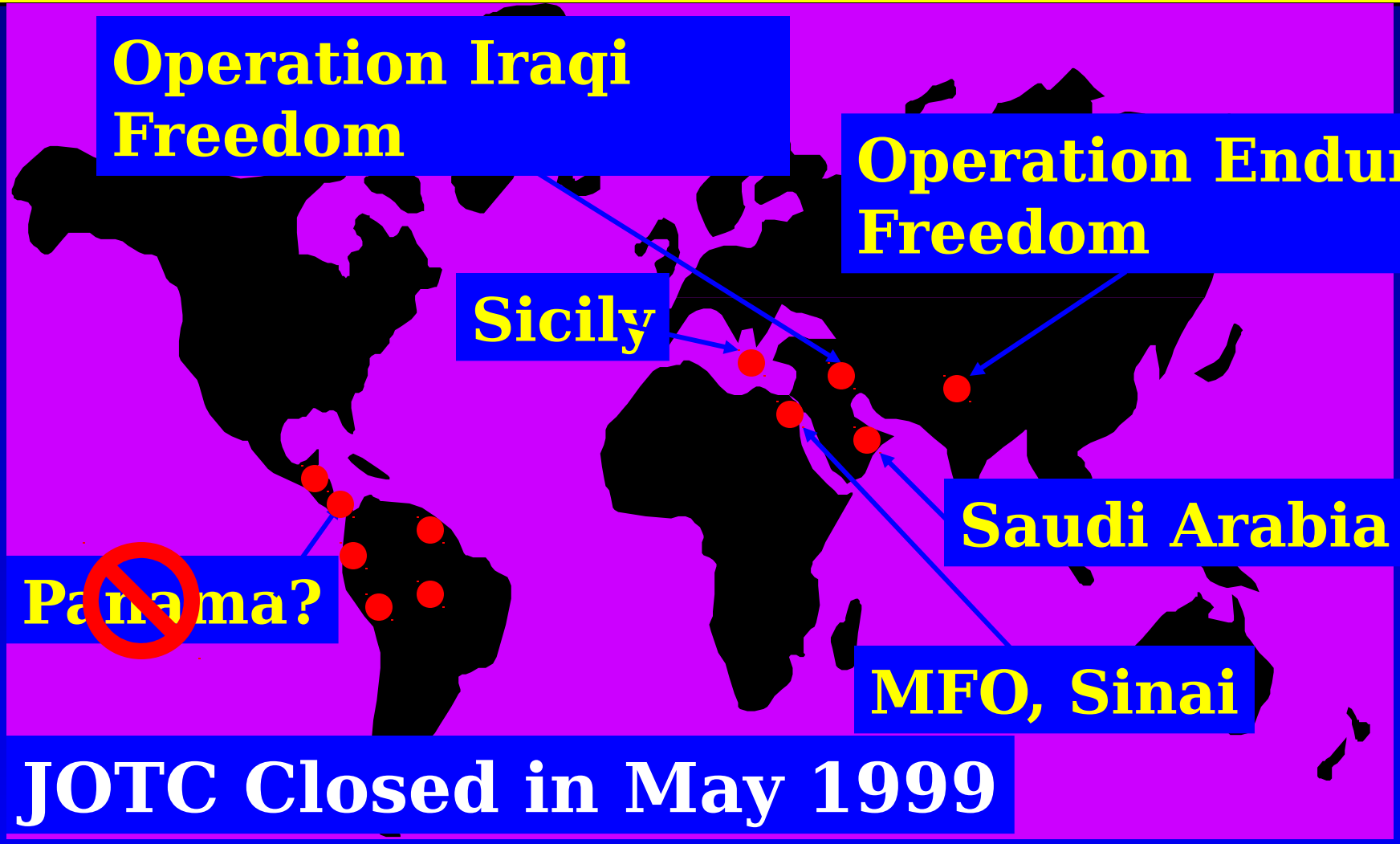
Day 0
Day 7
Day 14
Day 21

6 weeks
after Rx

6 months
after Rx
cm
1
2
3
4
5

Never attempt surgical excision!

Leishmaniasis Risk for US Military in 2006



NAS Sigonella

Leishmaniasis in Sicily

- **Naval Air Station Sigonella is located in Catania province, Sicily, an active focus of human VL**
- **30 - 40 cases of human VL per year in Italian hospitals since 1995.**
- **4 cases in US military dependent children since 1996**
- **Unknown number of “sub-clinical” infections**

Canine Leishmaniasis in Sicily

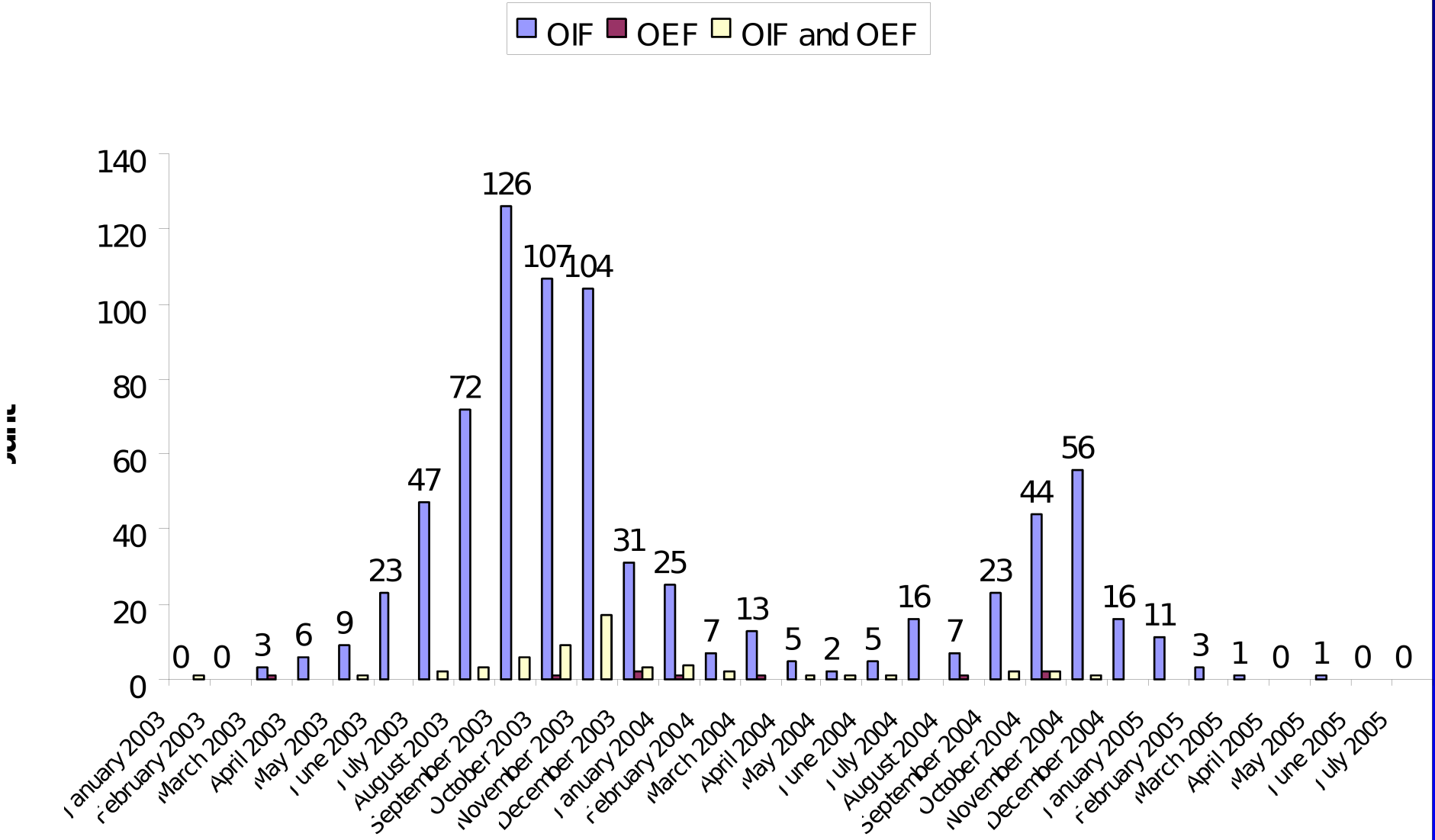
- Clinical and serologic testing of 50 companion dogs belonging to US military personnel
- 60% had increased antibody titers
- Inapparent infection in dogs is common
- Known sand fly vectors trapped in study area

Canine visceral leishmaniasis in Sicil
Mil Med 2000 Jan; 165(1): 29-32.

Leish, Iraq and OIF

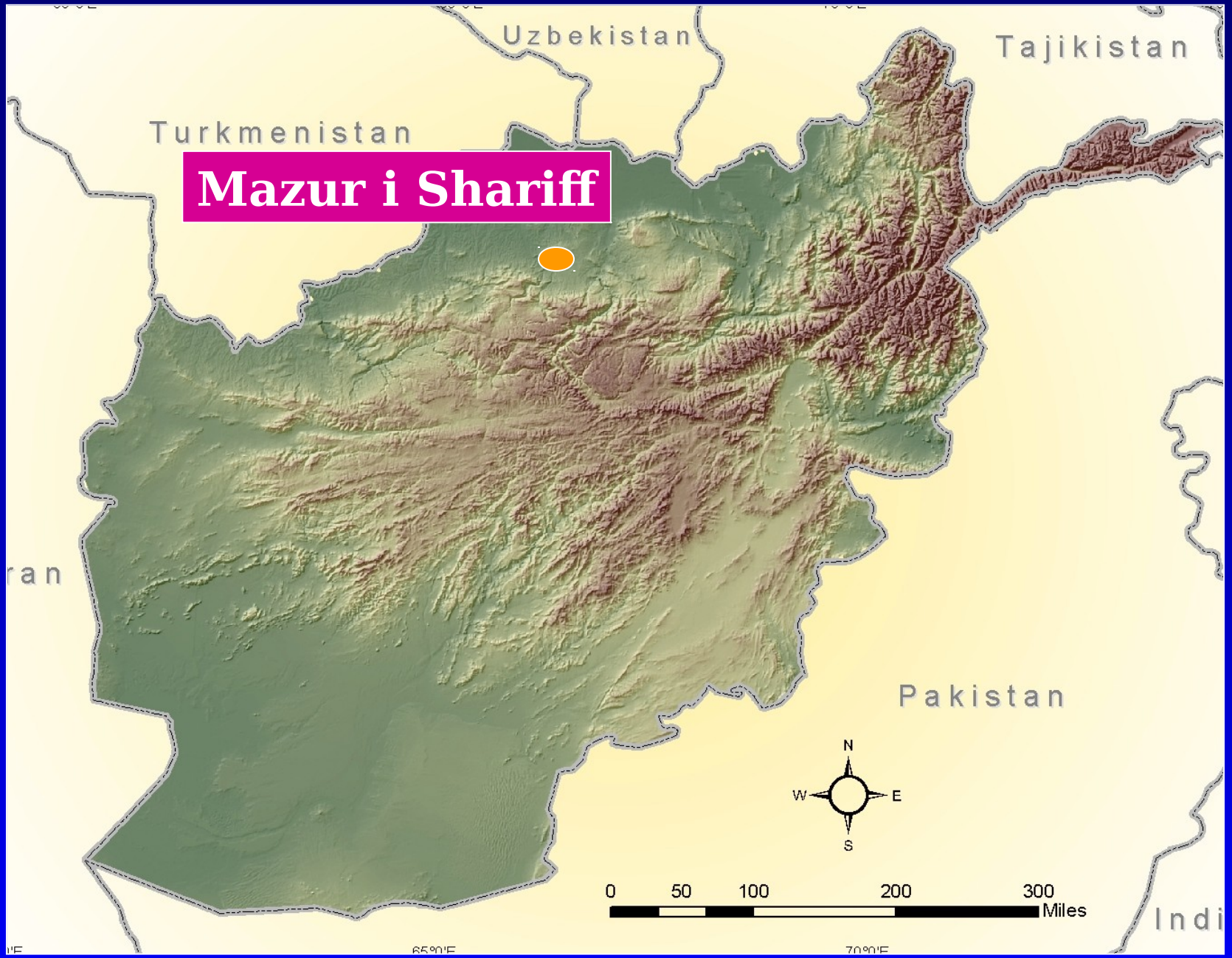
- **Cutaneous Disease**
  - **1500-2000+ cases**
  - **Virtually all from Iraq**
  - **Virtually all *L. major***
  - **Estimated 20K-30K lost duty days and a cost of >$10-12 million**
- **Visceral Disease**
  - **4 cases (2 Afghanistan, 2 Iraq)**
    - **1 case presented 14 months after leaving Afghanistan**
    - **Iraq case from the Green Zone**

Number of Leishmaniasis Cases* reported through RMES, by date symptom onset for confirmed cases, 1Jan '03 - 31Jul '05 Army (Active and Reserve Components) by Operation



Provided by LtCol Sean Moore, Army Medical Surveillance Activity

Dahuk
Dahuk
Bashur
Arbil
Irbil
CPA NORTH
Ninawa
Kirkuk
Sulaymaniyah
At Tamim
As Sulaymaniyah
IRAN
Hamadan
Dayr az Zawr
SYRIA
Salah ad Din
Euphrates R.
Tikrit
Diyala
Al Qaim
Samarra
CPA BAGHDAD
Baqubah
Ar Ramadi
Baghdad
Ar Rutbah
Fallujah
CPA SOUTH CENTRAL
Babil
Wasit
Al Hillah
Karbala
Al Anbar
Karbala
Al Kufa
Al Kut
Dezful
Al Amarah
An Najaf
Ad Diwaniyah
Al Qadisiyah
Maysan
As Samawah
Ahvaz
An Najaf
An Nasiriyah
Ar'ar
Dhi Qar
Al Basrah
CPA SOUTH
Az Zubayr
Al Basrah
Safwan
Umm Qasr
Al Muthanna'
SAUDI ARABIA
Rafha
Kuwait City
KUWAIT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OIF Diagnosis

- **Cutaneous Disease**
  - **Severe limitations with current diagnostic modalities**
    - **Culture (not widely available)**
    - **Microscopy (limited expertise, limited availability)**
- **<u>Solution</u>**
  - **Education of Army Pathologists via web-based learning**
  - **Polymerase Chain Reaction (PCR) assay mail out**

US Army special forces soldier
Presents to ‘TMC” in Bagram, Afghanistan
@ 3 week history of expanding ulcer on bac
1+ tender, not pruritic
What do you do??

OIF Chronic Ulcer Diff Dx

- **Cutaneous Leish**
- **Papule**
- **Asymptomatic**
- **Slower progression (week+), slowly ulcerates**
- **Rolled margin and periulcer induration**
- **No response to antibx**

- **Tropical pyoderma**
- **Pustule**
- **Pain & pruritus**
- **Rapid progression (days), pustule ruptures, ulcerates**
- **No rolled-up margin and no periulcer induration**
- **Responds to antibx**

Antibiotic Use

- **Suspect lesion**
  - **Augmentin 875 mg BID X 7-10 days**
  - **No response to Keflex**
- **Gets better or not**
- **Pursue PCR Dx for Leish if no response to antibiotics**

When to treat CL?

No Treatment		Treatment
Late* Early	*Stage of lesion(s)*	
Single (few) Multiple	*Number of lesions*	
Uncomplicated Complicated**	*Complexity*	
Immunocompetent Immunocompromised	*Immune status*	
None Yes	*Mucosal involvement*	
Non-exposed Exposed	*Skin location*	
Excluded Confirmed***	*L. braziliensis*	
No concern Very bothersome	*How bothersome are the lesions?*	
Small (<1 cm) large (> 5 cm)	*Size of lesion(s)*	Very

* By history, a lesion that is already starting to improve. It is "better" today than it was a few weeks ag
** Location of lesion(s) restricts joint mobility, prevents wearing of clothes or shoes, facial lesions may nose and ear lesions are very slow to heal.
*** confirmed *L. braziliensis* infections should be treated with parenteral SbV or amphotericin B deoxy

L. Major Treatment Options

- **Systemic Rx**
  - **Pentostam™ available on IND-status at WRAMC/BAMC**
  - **Amphotericin B deoxycholate (Fungizone)**
  - **Fluconazole**
- **Local Rx**
  - **ThermoMed™ device (heat Rx)**
  - **CryoRx (Dermatology)**
  - **Topical medications: paromomycin (not available)**
- **No treatment, natural healing**

OCT 16 2003

ThermoMed Device
Thermosurgery Inc, Phoenix AZ

- Portable, battery operated
- Delivers localized heat (50C X 30sec) via radiofrequency
- CDRH / FDA 510K cleared
- Device vs. Pentostam trial at WRAMC
- 23K per unit

MAR 8 2004

MAR 10 2004

MADE IN USA
MAR 12 2004

Therapeutic Outcome

ThermoMed

- **Randomized study at WRAMC, N=40, ulcerative *L. major* compare SSG to ThermoMed**
- **2 mo after treatment**
  - **73% cure with ThermoMed, easy to use**
  - **82% cure with 10 days SSG, significant toxicity**
- **~20% infection rate with ThermoMed**
  - **Topical antibiotic ointment after Rx prevents**
- **Cosmetic effect at 12 mo similar**

Data courtesy of COL Naomi Aronson

ThermoMed Devices in Iraq

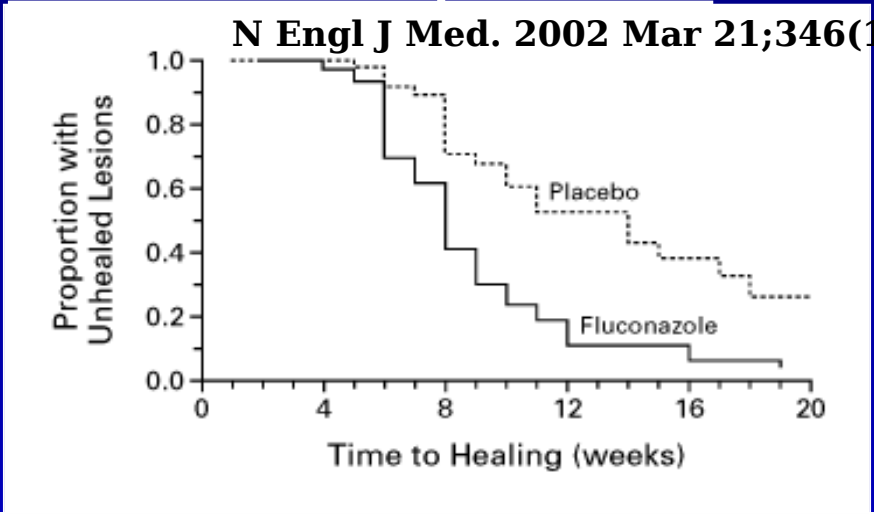
- **Mosul 47thCSH**
- **Tal Afar 3rd ACR clinic**
- **Baghdad Ibn Sina CSH**
- **Back-up device in Baghdad for other hot spots**

Cryotherapy

- **Widely used in the Middle East**
- **Poor quality trials to support use**
- **Little experience in USA with Leish**
- **Consider LN2 for small lesions, non-facial**
- **Dermatologists very familiar with LN2**

Fluconazole

Rationale / Data:



Recommendation:

- Do not recommend as firs
- Part of Rx toolbox
  - -occasional patients
  - -want Rx, don't want SS
  - -poor candidate for hea

Advantages:

- Oral route of administration
- Widely available
- Relatively safe
- Most MDs familiar with drug
- Could be used at BAS
- Only LFT medical monitoring required

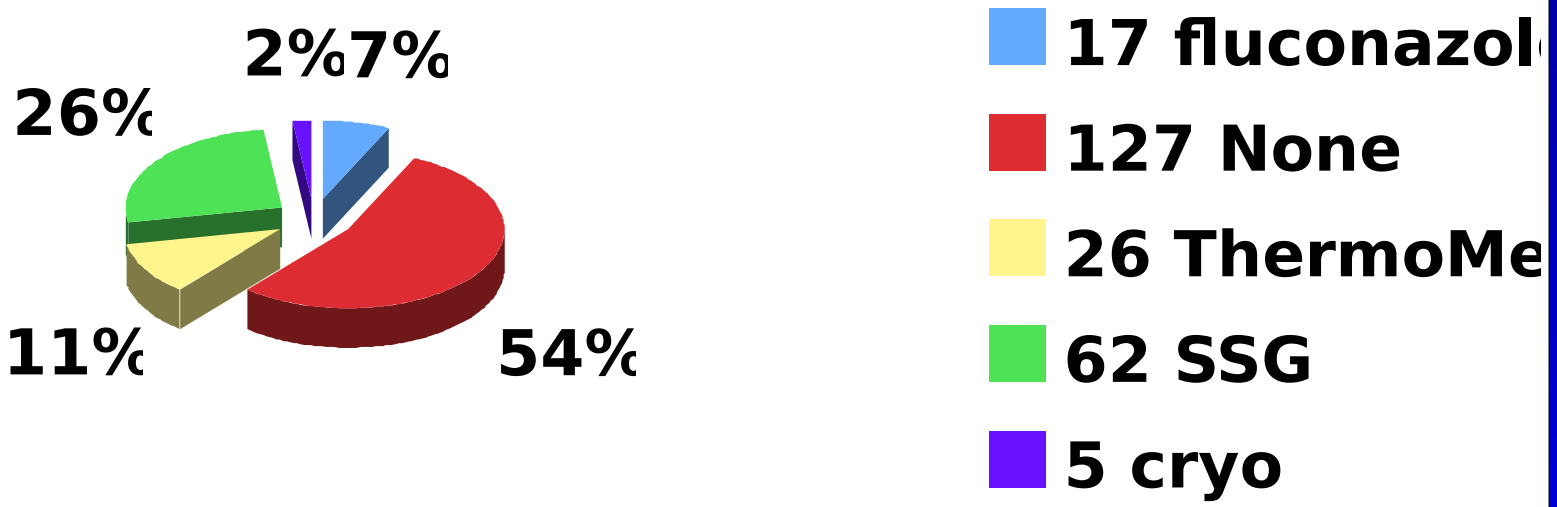
Limitations:

- Modestly efficacious
- Optimal dose and regime established
- Unless given DOT, effecti likely to be low
- No FDA indication for Le
- No data on *L. tropica*

Systemic CL Treatment Options

- **Sodium stibogluconate (Pentostam®)**
  - **Treatment IND**
  - **IND held by Surgeon General of the Army**
  - **WRAMC & BAMC currently only DoD Rx sites**
  - **20 days of IV therapy [20mg/kg/d]**
    - **10 days for *L. major* (with an investigator option for 20 days for facial, large or slow to heal lesions)**
  - **Patients treated as outpatients**

Distribution of Treatments
237 cases of CL



Data courtesy of MAJ Rob Willard and COL Doug Walsh

Ft. Campbell, KY OIF1

Prevention of Leishmaniasis

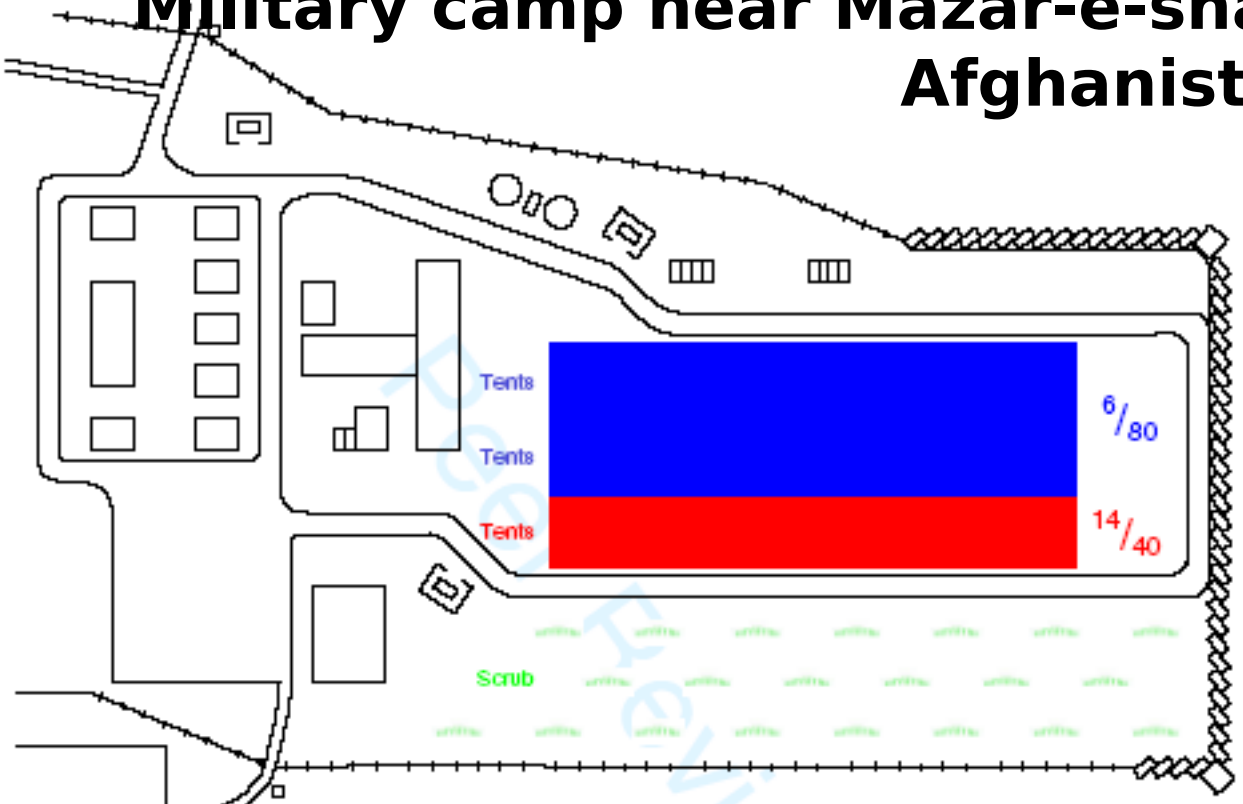
- **Know your enemy**
  - **Biology**
  - **Avoidance**
  - **PPMs**
  - **Where do you sleep?**

Prevention of Leishmaniasis

- Microhabitats lead to focal areas of high risk
- Sand flies are evening and night time biters
- Sandflies are poor fliers
  - Windy areas safer
  - No vegetation
- Air-conditioned sleeping quarters
- Impregnated bed nets
- Cleared compounds
- Rodent control
- Area or barrier insecticide use
- Education of the soldier and chain of command
- Use of a “knowledge card”

Importance of vegetation

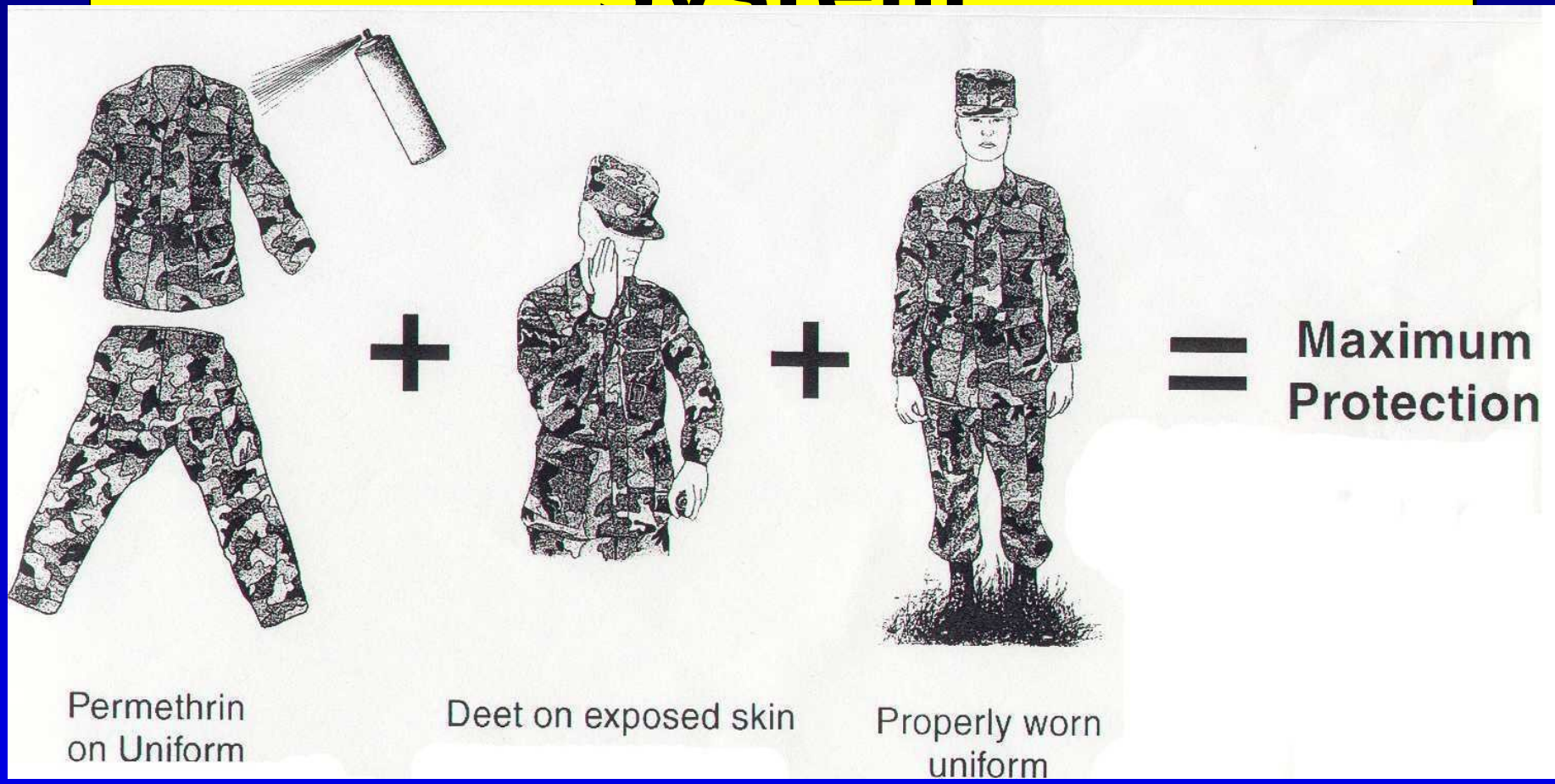
Military camp near Mazar-e-sharif, Afghanistan



Location, location, location!!

Peer Review

DoD Insect Repellent System



NSN 6840-01-278-1336
Permethrin Spray Can

NSN 6840-01-284-3982
DEET lotion

NSN 6840-01-345-0237
IDA Impregnation Kit

Any questions or consultation

Alan Magill, COL / MC
Walter Reed Army Institute of Research
301-319-9959 phone
alan.magill@na.amedd.army.mil